from various $A^{-}\cdot(M^{+})$ to PhSSPh vary with $E_R(A)$; the k_e values are correlated to the difference between $E_R(A)$ and $E_R(PhSSPh)$. Electron transfer from the free $A^{-}\cdot$ ion or the loose $A^{-}\cdot$ ion pair to PhSSPh was faster than that from the tight $A^{-}\cdot$ ion pair. Electron-withdrawing substituents of ArSSAr increase the electron-transfer rates or vice versa; these reflect the electron-acceptor abilities of the S–S bonds.

Experimental Section

Materials. Thioxanthone *S,S*-dioxide (TO–SO_2) was prepared by the method described in the literature.[49] Perylene (Pe), 1,12-benzoperylene (BPe), fluorenone (FO), and diaryl disulfides (ArSSAr) were purified by recrystallization and/or sublimation. Tetrahydrofuran (THF) and hexamethylphosphoric triamide (HMPA) were stored over the benzophenone ketyl or the pyrene radical anion and distilled before use on a high vacuum line. Arylthiolate anions (ArS^{-},M^{+}) were prepared from the corresponding disulfides upon contact with alkali metal in highly dried THF solution.

Methods. Slow decay kinetics for $(TO–SO_2)^{-}\cdot,M^{+}$ were followed with a Cary 14 spectrophotometer equipped with a temperature variable cell holder. Fast decay kinetics of $A^{-}\cdot(M^{+})$ were measured with a flash photolysis apparatus of a standard design; the half-duration of xenon flash lamps (Xenon Corp. N851) was ca. 10 μs and flash energy was ca. 100 J. Fluorescence was measured by a Shimazu RF501 fluorescence spectrophotometer. Electrochemical measurements of ArSSAr were made with cyclic volutammography (a Yanako P3V) with a Pt electrode in *N,N*-dimethylformamide solution containing tetra-*n*-butylammonium perchlorate as a supporting electrolyte.

Acknowledgment. We are indebted to Dr. T. Miyashita, Dr. K. Itaya, and Mr. M. Nakamura of Tohoku University for performing the electrochemical measurements and for their valuable comments.

(49) Ullman, F.; Lehner, A. *Ber. Dtsch. Chem. Ges.* **1905**, *38*, 729.

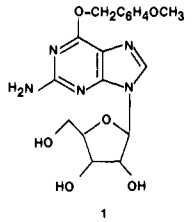
Dissociation of O^6-(*p*-Methoxybenzyl)guanosine in Aqueous Solution to Yield Guanosine, *p*-Methoxybenzylguanosines, and 4-(*p*-Methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole

Robert C. Moschel,* W. Robert Hudgins, and Anthony Dipple

Contribution from the Chemical Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland 21701. *Received March 5, 1981*

Abstract: The kinetics and products of O^6-(*p*-methoxybenzyl)guanosine (**1**) decomposition in MeOH/H_2O (5:95) at 40 °C have been examined over a wide range of pH values. The decomposition is acid catalyzed below pH 7, but over the pH range 8–13 the rate of decomposition is nearly pH independent. In addition to guanosine, N^2-(*p*-methoxybenzyl)guanosine, 4-(*p*-methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole, and 7-(*p*-methoxybenzyl)-, 1-(*p*-methoxybenzyl)-, and 8-(*p*-methoxybenzyl)guanosine are produced by the decomposition of **1**. Yields for these nucleoside products are pH dependent. An ionic mechanism involving dissociation of **1** to a *p*-methoxybenzylating agent which reassociates with either anionic and/or neutral guanosine largely accounts for the pH dependence of the product distributions.

As an extension of our previous studies of solvent and leaving-group effects on sites of benzylation of nucleic acid components,[1,2] we have initiated studies of the effects of para-substituent-induced changes in benzylating agent reactivity on the sites of benzylation of guanosine under neutral aqueous conditions.[3] These investigations required preparation of a series of para-substituted benzylguanosine derivatives for product stability studies and for use as chromatographic marker compounds. We observed that, of the several O^6-substituted guanosines we prepared, O^6-(*p*-methoxybenzyl)guanosine (**1**) is markedly unstable in aqueous media and quite unexpectedly undergoes transformation to a variety of nucleoside products. We present here details of the kinetics and products of these novel reactions.



1

Results

Preparation of O^6-(*p*-methoxybenzyl)guanosine (**1**) was patterned after Gerster and Robins'[4] procedure for preparation of O^6-benzylguanosine. While this latter compound can be purified by crystallization from water, our attempts to purify **1** by this method led only to the recovery of guanosine as a precipitated solid. The lability of O^6-(*p*-methoxybenzyl)guanosine required alternative procedures for its purification (see Experimental Section) and also prompted our investigation of the kinetics of its decomposition in largely aqueous medium.

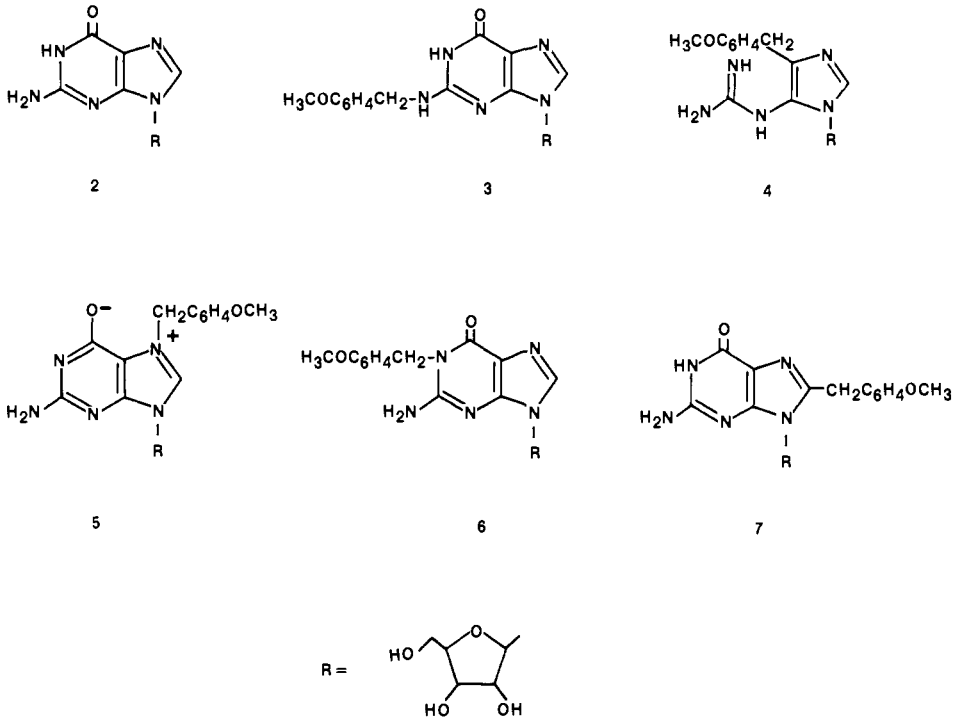
Rates of disappearance of **1** in MeOH/H_2O (5:95) at 40 °C were determined spectrophotometrically by monitoring the decrease in absorbance of solutions of **1** at 280 nm as a function of time. Plots of ln $(OD_t - OD_\infty)$ vs. time were linear for at least 3 to 4 half-times indicating that the decomposition is first order in **1**. Values for the observed first-order rate constants (k_{obsd}) were calculated from the slopes of these plots.

The magnitude of k_{obsd} was found to be pH dependent, and a plot of log k_{obsd} vs. pH is shown in Figure 1. Over the pH range 7–5.5, Figure 1 illustrates that k_{obsd} increases with decreasing pH; the slope of the log k_{obsd} vs. pH plot is approximately −1 in this pH range, indicating that the decomposition of **1** is acid catalyzed below pH 7. However, at pH values greater than pH 8, the rate of decomposition of **1** is nearly pH independent. From least-squares plots of k_{obsd} vs. hydrogen ion activity, it was determined that the experimental values of k_{obsd} could be satisfactorily fit to

(1) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1979**, *44*, 3324.
(2) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *J. Org. Chem.* **1980**, *45*, 533.
(3) Moschel, R. C.; Hudgins, W. R.; Dipple, A. *Tetrahedron Lett.* **1981**, *22*, 2427.
(4) Gerster, J. F.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 3752.

0002-7863/81/1503-5489$01.25/0

Chart I



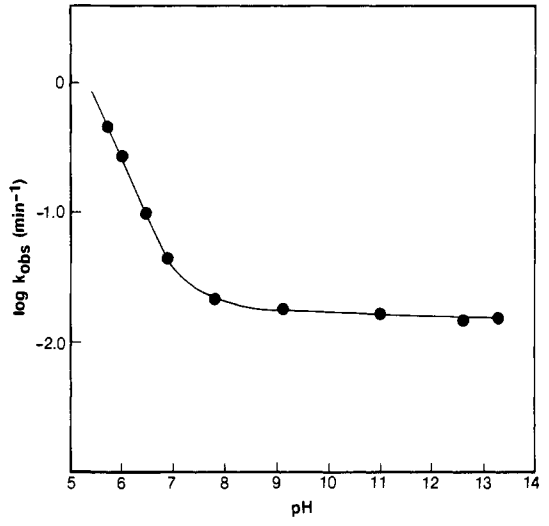


Figure 1. Plot of log k_{obsd} vs. pH for the disappearance of **1** in MeOH/H_2O (5:95), 40 °C.

the two-term expression, $k_{obsd} = k_H{}^+a_H{}^+ + k_0$, where $k_H{}^+ = 2.27 \times 10^5$ M^{-1} min^{-1} and $k_0 = 1.93 \times 10^{-2}$ min^{-1}.

When the decomposition of **1** is complete, the UV spectrum of the kinetic solutions resembles the spectrum of guanosine (**2**) as would be expected for a hydrolytic cleavage of the O^6-ether linkage. However, chromatographic fractionation of more concentrated decomposition solutions revealed the presence of at least five other nucleosides in addition to guanosine, indicating that some novel rearrangements or dissociation/reassociation phenomena had occurred. These additional products were identified as N^2-(*p*-methoxybenzyl)guanosine (**3**), 4-(*p*-methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**4**), 7-(*p*-methoxybenzyl)guanosine (**5**), 1-(*p*-methoxybenzyl)guanosine (**6**), and 8-(*p*-methoxybenzyl)guanosine (**7**). The identity of each of these adducts was confirmed by comparison of its column-chromatographic and UV-spectroscopic properties with those of a fully characterized reference compound prepared under different experimental conditions. The 7-substituted product (**5**) was prepared by reaction of *p*-methoxybenzyl bromide with guanosine in *N*,*N*-dimethylformamide (DMF) under conditions similar to those employed earlier for preparation of 7-benzylguanosine.[5] Products **3**, **4**, **6**, and **7** were isolated from a large-scale guanosine–*p*-methoxybenzyl chloride reaction carried out in alkaline (Na_2CO_3) aqueous solution. Under these conditions the yield for product **3** is similar to that in neutral aqueous solution, but the yields for **4** and **7** were at least sevenfold greater, and for **6** the yields were nearly 16 times greater than in neutral aqueous solution.[6]

The site of attachment of the *p*-methoxybenzyl residue in products **6** and **7** was readily ascertained from their UV and NMR spectral characteristics which resembled those reported for known 1-[7] and 8-benzylguanine[8] derivatives. Confirmation of structure **3** was provided by its NMR spectrum in Me_2SO-d_6 which shows a two-proton distorted doublet for the benzylic hydrogens at δ 4.47 which is superimposed on the multiplet for the H-2′ of the ribose moiety. The chemical shift and observed multiplicity for the benzylic hydrogens of N^2-benzylguanosine[1] were very similar to those for **3**. For the former compound, the peak for the single exchangeable hydrogen attached to the amino group of the guanine moiety appeared as a triplet at δ 6.92 (J = 6 Hz). Although a similar multiplet for a single exchangeable proton is present in the NMR spectrum of **3** at δ ~6.9, this multiplet lies beneath the two farthest upfield peaks which comprise the four-proton quartet (δ 7.13) for the aromatic protons of the *p*-methoxybenzyl residue. On addition of D_2O, the multiplet beneath these peaks disappears and the distorted doublet for the benzylic hydrogens of **3** resembles a two-proton singlet. These features of the NMR spectrum clearly confirm that the exocyclic amino group of guanosine is the site of attachment of the *p*-methoxybenzyl group in product **3**.

Probably the most striking aspect of the reaction of guanosine with *p*-methoxybenzyl chloride in aqueous Na_2CO_3 solution is the formation of 4-(*p*-methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**4**) as the *major* product of reaction. This material is an analogue of the *p*-methylbenzyl-substituted nucleoside detected in the reaction of *p*-methylbenzyl chloride with guanosine in neutral aqueous solution.[3] As observed for the *p*-methylbenzyl analogue,[3] solutions of **4** exhibit only weak ab-

(5) Brookes, P.; Dipple, A.; Lawley, P. D. *J. Chem. Soc.* **1968**, *2026*.
(6) Unpublished results from this laboratory.
(7) Philips, K. D.; Horwitz, J. P. *J. Org. Chem.* **1975**, *40*, 1856.
(8) Christensen, L. F.; Meyer, R. B.; Miller, J. P.; Simon, L. N.; Robins, R. K. *Biochemistry* **1975**, *14*, 1490.

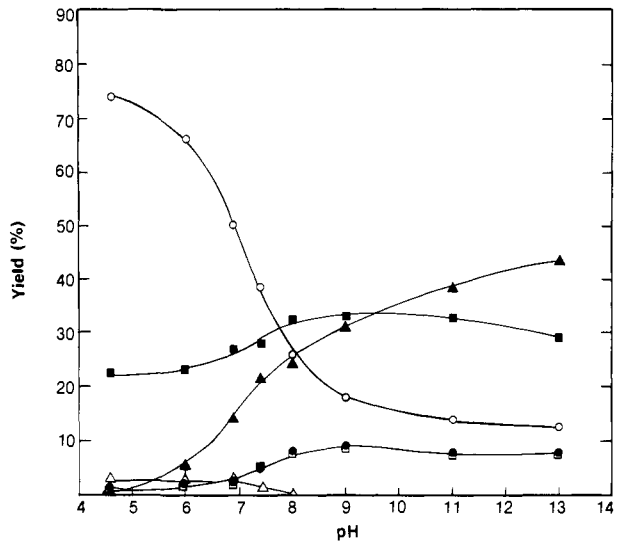


Figure 2. Plot of % yield vs. pH for products **2–7** derived from the decomposition of **1** in $MeOH/H_2O$ (5:95), 40 °C: product **2**, ○; **3**, ■; **4**, ▲; **5**, △; **6**, □; **7**, ●. Yields are reported as a percentage of total products.

sorption in the 240–290-nm region of the ultraviolet at either pH 6.9 or pH 1 and show a maximum at 274 nm assignable to the *p*-methoxybenzyl chromophore. In alkaline solution, the spectrum of **4** shows essentially only more intense end absorption in the 240–290-nm region. The NMR spectra for **4** and those of the *p*-methylbenzyl analogue[3] are essentially identical except for the expected differences in chemical shifts for hydrogens attached to the respective para-substituted benzene rings. Similarly, the fragmentation pattern in the mass spectra for both compounds are essentially identical. The *m/z* ratio for fragment ions generated from **4** differ from those of the *p*-methylbenzyl analogue[3] by the expected 16 amu. Thus, the reaction of guanosine with either *p*-methylbenzyl or *p*-methoxybenzyl chloride produces an analogous imidazole nucleoside.

When the yields for each of the nucleosides **2–7** arising from the decomposition of **1** were measured, some interesting variations with pH were apparent (Figure 2). At pH 4.6, guanosine and N^2-(*p*-methoxybenzyl)guanosine are the major products of the decomposition of **1** and are formed in 74 and 22% yields, respectively. Low yields of 7-, 8-, and 1-(*p*-methoxybenzyl)guanosine (**5**, **7**, and **6**) are produced, and 4-(*p*-methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**4**) is not detected at this pH. An increase in pH to 7.4 is accompanied by decreased guanosine (**2**) liberation, slight increases in the yields for the N^2-, 8-, and 1-substituted derivatives (**3**, **7**, **6**), and a dramatic increase in the yield of **4**, such that yields for **2**, **3**, **4**, **6**, **7**, and **5** are 39, 28, 22, 5, 5, and 1%, respectively. Increasing the pH to 13 is accompanied by still further decreases in guanosine liberation and the 7-substituted derivative (**5**) is not detected above pH 8 either as the intact substituted purine derivative or as the imidazole ring-opened form. The yields for adducts **3**, **7**, and **6** are not substantially affected by changes in pH in the range pH 9–13. However, the extent of formation of **4** continues to increase in the alkaline pH range, and at both pH 11 and 13 it is the *major* product of O^6-(*p*-methoxybenzyl)guanosine decomposition.

Overall, it appears that the decreasing yield of guanosine with increasing pH is balanced by increasing yields of *p*-methoxybenzylated nucleosides and, with the possible exception of the reaction carried out at pH 13, the total recoveries for all products do not suggest significant instability of any major product over the range of pH used for the kinetic measurements. Failure to detect the intact N-7 substituted product or the imidazole ring-opened derivative at pH 8 and above might suggest instability of these products under these conditions. The ever-increasing yield of **4** with increasing pH might suggest that one step in the formation of this imidazole nucleoside is base catalyzed. This could not be a rate-determining step in the decomposition of **1**, however, since this rate of decomposition is nearly pH independent in the alkaline range (Figure 1).

Table I. Approximate Rate Constants for Formation of Products **2–7** from the Decomposition of **1** in $MeOH/H_2O$ (5:95), 40 °C[a]

product	k_{H^+}, M^{-1} min^{-1}	k_0, min^{-1}
2	1.6×10^5	2.7×10^{-3}
3	5.1×10^4	6.3×10^{-3}
4	1.1×10^4	7.4×10^{-3}
5	4.5×10^3	~0
6	1.1×10^3	1.4×10^{-3}
7	1.4×10^3	1.4×10^{-3}

[a] Rate constants were estimated from k_{obsd} for the disappearance of **1** and the fractional yields for the individual products.

The data in Figure 2 can also be described, of course, by estimating (from k_{obsd} for the disappearance of **1** and individual product yields) the observed first-order rate constants for formation of each product at each pH value.[9] Plots of these vs. pH for the individual products over the pH range 5.5–13 reveal that the general shape of these plots for all products except **5** resembles that for the disappearance of **1** (Figure 1). Thus k_{obsd} for formation of each product other than **5** would be described by a two-term expression $k_{obsd} = k_H{}^+a_H{}^+ + k_0$. Since formation of **5** is not detected in reactions carried out at pH ≥8, plots of log k_{obsd} vs. pH for this product decrease sharply over the pH range 5.5–8, and consequently the expression for k_{obsd} for formation of this product would presumably contain a negligibly small k_0 term. Approximate values for $k_H{}^+$ and k_0 which satisfactorily account for the observed product distributions over the pH range 6–13 are presented in Table I.

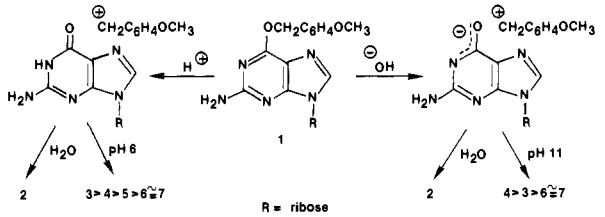
Discussion

Although it is well known that anionic guanine derivatives are substantially more reactive toward alkylating agents than the corresponding neutral forms, the reactivity of guanosine toward aralkylating agents in alkaline aqueous solution has not been widely studied. Lyle et al.[10] have recently documented the enhanced reactivity of guanosine anion toward 6-chloromethylbenzo[*a*]-pyrene in acetone/H_2O (1:1), and in dioxane/aqueous base (1:1) they detected formation of 1- and O^6-substituted guanosines. Kasai et al.[11] and Nakanishi et al.[12] characterized guanosine substituted at the O-2′ and 8 position after reaction with 7,12-dimethylbenz[*a*]anthracene 5,6-oxide in alkaline aqueous acetone solution. From similar synthetic reactions in totally aqueous solvent we were able to obtain 1-, N^2-, and 8-(*p*-methoxybenzyl)guanosine in addition to product **4**. Despite the low yields, the difficulties of more complex synthetic schemes make this one-step reaction a convenient route to these new nucleosides.

The high instability of the O^6-aralkyl ether linkage in **1** was not altogether expected. While it is known that the O^6-alkyl ether linkage in O^6-methyl- or O^6-ethylguanine is acid labile, it is sufficiently stable to survive conditions of temperature and acidity required to liberate purine bases from DNA. **1** is clearly far more sensitive to acid than the alkyl analogues, and, in addition, it decomposes at a slower but still substantial rate in the absence of any acid catalysis ($t_{1/2}$ at pH 9, 37 min) (Figure 1). It is far less stable than O^6-benzylguanosine in aqueous solution,[1,4] indicating that the O^6-aralkyl ether linkage is labilized by electron-donating para substituents on the benzene ring as would be expected for a reaction involving an ionic transition state with significant positive charge development at the benzylic carbon center.

(9) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism", 2nd ed.; Wiley: New York, 1961; Chapter 8.
(10) Lyle, T. A.; Royer, R. E.; Daub, G. H.; Vander Jagt, D. L. *Chem.-Biol. Interact.* **1980**, *29*, 197.
(11) Kasai, H.; Nakanishi, K.; Frenkel, K.; Grunberger, D. *J. Am. Chem. Soc.* **1977**, *99*, 8500.
(12) Nakanishi, K.; Komura, H.; Miura, I.; Kasai, H. *J. Chem. Soc., Chem. Commun.* **1980**, 82.

Scheme I



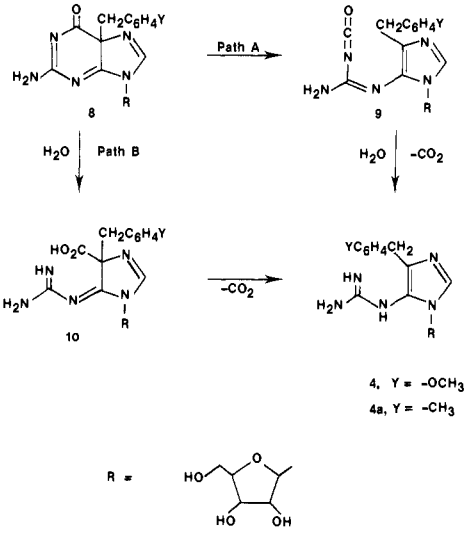
The involvement of an ionic transition state is also suggested by the apparent solvent dependence for the decomposition of **1**; i.e., this material is stable for at least 3 days at 25 °C in MeOH/H_2O (1:1), and it can be recrystallized from hot MeOH with little decomposition, and by the similarity between product distributions from the decomposition of **1** and those obtained by reaction of guanosine with *p*-methoxybenzyl chloride. Thus, treatment of radiolabeled guanosine at pH 4.5 with *p*-methoxybenzyl chloride produces products **3**, and **5–7** in virtually the same ratio (see Experimental Section) as is produced in the decomposition of **1** at the same pH (Figure 2) although the yields for the chloride reaction are far lower. Similarly, the product distribution observed in the preparative-scale reaction of guanosine anion with *p*-methoxybenzyl chloride (Experimental Section) is very similar to that observed in the decomposition of **1** carried out at alkaline pH (pH 11–13, Figure 2), although, again, the yields with the chloride are substantially lower.

The decomposition of **1** to the nucleoside products described herein does not seem to be analogous to the rearrangements recently described by Leonard and co-workers[13-16] for guanine substituted at the O^6 position by allylic alkyl groups. These workers found that such compounds undergo rearrangement in high yield to the corresponding 8-substituted base through an intramolecular mechanism involving two [3,3] sigmatropic shifts via C-5. These rearrangements are facilitated by formation of the anion of the O^6-allyl-substituted base and take place in nonaqueous solvents at high temperature. Significantly, the anion of O^6-benzylguanine does not undergo this type of sigmatropic rearrangement, but forms instead N^2-benzylguanine.[13]

Taken together, all these considerations lead us to propose an ionic mechanism for the decomposition of **1** involving a preliminary dissociation of starting material to a cationic *p*-methoxybenzylating agent and a guanosine leaving group (Scheme I). Trapping the intermediate aralkylating agent with water leads to liberation of **2** as a solvolysis product, while formation of products **3–7** can be readily envisaged as a consequence of "leaving group return". In the strongly alkaline pH range, dissociation of **1** will provide an intermediate ion pair which reassociates to give products characteristic of the reaction of guanosine anion with a *p*-methoxybenzylating agent. Since the reactivity of guanosine anion is markedly enhanced with respect to that of the neutral species, reassociation giving **3**, **4**, **6**, and **7** is favored over solvolysis. In more acidic medium, where the decomposition of **1** is acid catalyzed, protonation of **1** must precede dissociation which then gives rise to an aralkylating species and neutral guanosine as leaving group. Leaving group return is then less efficient, and solvolysis of **1** to liberate **2** and *p*-methoxybenzyl alcohol predominates. At intermediate pH values, product distributions reflect the complex reassociation of the *p*-methoxybenzyl group with both the anionic and neutral forms of the guanosine leaving group.

The detection of product **4** provides the second account of formation of nucleosides of this type as products of guanosine aralkylation in aqueous solution. The simplest mechanism for formation of **4** and the previously described **4a**[3] is illustrated in Scheme II and probably involves intermediate formation of a 5-substituted guanosine (**8**), followed by either an electrocyclic rearrangement to **9** and subsequent hydrolysis and loss of CO_2 (path A, Scheme II) or by hydrolytic cleavage of the pyrimidine ring to **10** followed by decarboxylation (path B, Scheme II). While

Scheme II



adducts such as **8** have not yet been isolated as products of reaction with guanosine, similar structures have been proposed as intermediates[13-16] (see above), and Jefcoate et al.[17] have observed both alkylation and aralkylation at an analogous carbon atom of a dihydroalloxazine. Derivatives such as **9** have also not been isolated but Holtwick et al.[18] have proposed the intermediacy of similar species in certain rearrangements.

The marked instability of O^6-(*p*-methoxybenzyl)guanosine in neutral aqueous solution suggests that other O^6-substituted guanine derivatives might be similarly unstable. For example, while there are no reports of the modification of the O^6 position of guanine residues by solvolytically reactive derivatives of the polycyclic aromatic hydrocarbon carcinogens under neutral aqueous conditions, the present report suggests that, if such adducts were formed, they would probably be hydrolytically unstable and might undergo transformation to products analogous to **3–8** as described herein.

While the major sites of reaction between such reactive hydrocarbon derivatives and nucleic acids both in vitro and in vivo are known to be the amino groups of the nucleic acid bases,[19-28] it is not inconceivable that biological effects could be elicited by minor products of the type we have described arising from transiently formed O^6-substituted guanine residues.

Experimental Section

Ultraviolet absorption spectra were recorded on a Cary 17 spectrophotometer. Kinetic measurements were carried out on a Gilford Model 250 spectrophotometer equipped with a temperature-controlled cell compartment maintained at 40 ± 0.3 °C. NMR spectra were recorded on a Varian XL 100 spectrometer operated in the Fourier transform

(13) Frihart, C. R.; Leonard, N. J. *J. Am. Chem. Soc.* **1973**, *95*, 7174.
(14) Leonard, N. J.; Frihart, C. R. *J. Am. Chem. Soc.* **1974**, *96*, 5894.
(15) Holmes, B. N.; Leonard, N. J. *J. Org. Chem.* **1976**, *41*, 568.
(16) Holmes, B. N.; Leonard, N. J. *J. Org. Chem.* **1978**, *43*, 516.
(17) Jefcoate, C. R.; Ghisla, S.; Hemmerick, P. *J. Chem. Soc. C* **1971**, 1689.
(18) Holtwick, J. B.; Golankiewicz, B.; Holmes, B. N.; Leonard, N. J. *J. Org. Chem.* **1979**, *44*, 3835.
(19) Dipple, A.; Brookes, P.; Mackintosh, D. S.; Rayman, M. P. *Biochemistry* **1971**, *10*, 4323.
(20) Jeffrey, A. M.; Blobstein, S. H.; Weinstein, I. B.; Beland, F. A.; Harvey, R. G.; Kasai, H.; Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2311.
(21) Jeffrey, A. M.; Grezeskowiak, K.; Weinstein, I. B.; Nakanishi, K.; Roller, P.; Harvey, R. G. *Science* **1979**, *206*, 1309.
(22) Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Weinstein, I. B.; Beland, F. A.; Harvey, R. G.; Kasai, H.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1976**, *98*, 5714.
(23) Koreeda, M.; Moore, P. D.; Yagi, H.; Yeh, H. J. C.; Jerina, D. M. *J. Am. Chem. Soc.* **1976**, *98*, 6720.
(24) Meehan, T.; Straub, K.; Calvin, M. *Nature (London)* **1977**, *269*, 725.
(25) Osborne, M. R.; Beland, F. A.; Harvey, R. G.; Brookes, P. *Int. J. Cancer* **1976**, *18*, 362.
(26) Rayman, M. P.; Dipple, A. *Biochemistry* **1973**, *12*, 1202.
(27) Rayman, M. P.; Dipple, A. *Biochemistry* **1973**, *12*, 1538.
(28) Shapiro, R.; Shiuey, S. J. *J. Org. Chem.* **1976**, *41*, 1597.

mode at 100 MHz. Samples were dissolved in dimethyl-d_6 sulfoxide using 0.5% tetramethylsilane as internal standard. Mass spectra were obtained on a Finnigan 3300 mass spectrometer equipped with a Finnigan 6000 MS data system. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

p-Methoxybenzyl alcohol and 2-amino-6-chloropurine riboside were obtained commercially. *p*-Methoxybenzyl chloride[29] and bromide[30] were prepared from *p*-methoxybenzyl alcohol by treatment with gaseous HCl or HBr followed by vacuum distillation.

O^6-(*p*-Methoxybenzyl)guanosine (1). This material was prepared through reaction of sodium *p*-methoxybenzylate (0.8 g) with 2-amino-6-chloropurine riboside (0.25 g) in 5 mL of molten *p*-methoxybenzyl alcohol following the method of Gerster and Robins.[4] After 25 min at 100 °C, the crude product was precipitated by pouring the reaction solution into a large excess of ether. No attempt was made to neutralize the alkaline suspension with acetic acid. The crude precipitate was collected by filtraton and redissolved in 40 mL of MeOH/H_2O/concentrated NH_3–H_2O (5:5:0.3) and was loaded on a 2.8 × 71 cm Sephadex LH-20 column. The column was eluted with this same solvent (flow rate: 1 mL/min) and fractions (10 mL) were collected. UV absorption was continuously monitored at 254 nm. O^6-(*p*-Methoxybenzyl)guanosine (**1**) eluted in fractions 87–110. These fractions were pooled and were diluted with 800 mL of 2-propanol; the resulting solution was evaporated to dryness under reduced pressure at 40 °C. The resulting residue was crystallized from a small volume of MeOH to afford 0.15 g (45%) of **1**: UV λ_{max} (pH 10) 246 nm (ϵ 9.98 × 10^3), 279 (1.08 × 10^4); (pH 13) 246 (9.87 × 10^3), 279 (1.12 × 10^4); NMR δ 3.58 (m, 2, H-5′), 3.77 (s, 3, OCH_3), 3.88 (m, 1, H-4′), 4.10 (m, 1, H-3′), 4.46 (m, 1, H-2′), 5.10 (distorted t, 2, OH-5′ + OH, exchange with D_2O), 5.38 (d, 1, OH, exchanges with D_2O), 5.46 (s, 2, $ArCH_2$), 5.80 (d, 1, H-1′), 6.48 (s, 2, NH_2, exchange with D_2O), 7.22 (q, 4, Ar), 8.11 (s, 1, H-8). Anal. Calcd for $C_{18}H_{21}N_5O_6$: C, 53.59; H, 5.25; N, 17.36. Found: c, 53.33; H, 5.35; N, 17.11.

7-(*p*-Methoxybenzyl)guanosine (5). To a suspension of 0.6 g of guanosine in 25 mL of DMF was added 2 mL of *p*-methoxybenzyl bromide, and the resulting solution was stirred at room temperature for 20 h. Crude product (0.40 g) was precipitated by the addition of 300 mL of ether. A portion of this material was purified by dissolving 0.2 g of the crude product in 40 mL of acetone and sufficient MeOH to achieve complete dissolution. This solution was treated with 1 equiv of NH_3–H_2O. A voluminous precipitate formed immediately and was filtered and washed with ether and air-dried; 0.10 g of this solid was suspended in 30 mL of warm MeOH/H_2O (3:7) with stirring until no further dissolution of solid was apparent. The suspension was filtered and the filtrate was loaded on a 2.8 × 71 cm Sephadex LH-20 column eluted with MeOH/H_2O (3:7) (flow rate 1 mL/min). UV absorption was continuously monitored at 254 nm and fractions (10 mL) were collected. Product **5** (0.026 g) eluted in homogeneous form in fractions 28–32: UV λ_{max} (pH 1) 257 nm (ϵ 1.28 × 10^4), 280 (sh) (8.03 × 10^3); (pH 6.9) 258 (1.04 × 10^4), 285 (7.25 × 10^3); (pH 13) 266 (1.03 × 10^4) (these latter spectral data are for the anion of the imidazole ring-opened form); NMR δ 3.70 (m, 2, H-5′), 3.76 (s, 3, OCH_3), 4.02 (m, 1, H-4′), 4.14 (m, 1, H-3′), 4.42 (m, 1, H-2′), 5.34 (m, 2, OH-5′ + OH, exchange with D_2O), 5.57 (s, 2, $ArCH_2$), 5.70 (broad s, 1, OH, exchanges with D_2O), 5.85 (d, 1, H-1′), 7.23 (q, 4, Ar), 7.56 (s, 2, NH_2, exchange with D_2O), 9.77 (s, 1, H-8), 12.04 (s, 1, 1-NH, exchanges with D_2O). Anal. Calcd for $C_{18}H_{22}N_5O_6 \cdot Br \cdot {}^1/_2H_2O$: C, 43.82; H, 4.70; N, 14.19. Found: C, 43.89; H, 4.72; N, 13.94.

8-(*p*-Methoxybenzyl)guanosine (7), 1-(*p*-Methoxybenzyl)guanosine (6), N^2-(*p*-Methoxybenzyl)guanosine (3), and 4-(*p*-Methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (4). To a warm solution of guanosine (6 g, 21 mmol) in 200 mL of H_2O containing 9 g of Na_2CO_3 (85 mmol) was added 10 g (64 mmol) of *p*-methoxybenzyl chloride. The heterogeneous solution was stirred vigorously for 30 min by which time the pH of the reaction solution had decreased to pH ~9 and unreacted guanosine began to precipitate. The mildly alkaline solution was twice extracted with 300 mL of $CHCl_3$. The pH of the remaining aqueous phase was adjusted to pH 5.5 with dilute HCl and stirring until effervescence ceased. The resulting suspension was filtered and the filtrate was evaporated to dryness. The dry residue was extracted with 100 mL of MeOH, the suspension was filtered, and the filtrate was again evaporated to dryness. The remaining dry residue was redissolved in 50 mL of MeOH/H_2O (3:7) and made alkaline by the addition of 1.5 mL of NH_3–H_2O. This solution was loaded on a 2.8 × 71 cm Sephadex LH-20 column which was eluted with MeOH/H_2O/NH_3–H_2O (3:7:0.3) at 1 mL/min. Fractions (10 mL) were collected and UV absorption was continuously monitored at 254 nm. Unreacted guanosine (**2**) eluted in fractions 27–35, 8-(*p*-methoxybenzyl)guanosine (**7**) eluted in fractions 35–41, N^2-(*p*-methoxybenzyl)guanosine (**3**) eluted in fractions 42–50, 4-(*p*-methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**4**) eluted in fractions 51–60, and 1-(*p*-methoxybenzyl)guanosine (**6**) eluted in fractions 80–100. Since products **7**, **3**, and **4** were not completely resolved, the appropriate fractions rich in the individual products were rechromatographed under identical chromatographic conditions. Rechromatography of pooled fractions 35–41 afforded 0.06 g (0.7%) of **7**. An analytical sample was prepared by crystallization from aqueous 1% KCl solution: UV λ_{max} (pH 1) 262 nm (ϵ 1.92 × 10^4), 275 (sh) (1.50 × 10^4); (pH 6.9) 255 (2.14 × 10^4), 272 (sh) (1.58 × 10^4); (pH 13) 265 (1.82 × 10^4); NMR δ 3.59 (m, 2, H-5′), 3.74 (s, 3, OCH_3), 3.80 (m, 1, H-4′), 4.08 (s, 2, $ArCH_2$), 4.10 (m, 1, H-3′), 4.70 (m, 1, H-2′), 5.18 (br m, 3, OH-5′, OH-2′, OH-3′, exchange with D_2O), 5.70 (d, 1, H-1′), 6.29 (s, 2, NH_2, exchange with D_2O), 7.02 (q, 4, Ar), 10.56 (broad s, 1, 1-NH, exchanges with D_2O). Anal. Calcd for $C_{18}H_{21}N_5O_6 \cdot 2H_2O$: C, 49.20; H, 5.73; N, 15.94. Found: C, 49.16; H, 5.75; N, 15.71.

Rechromatography of pooled fractions 42–50 afforded 0.37 g (4%) of **3**. An analytical sample was prepared by crystallization from aqueous 1% KCl: UV λ_{max} (pH 1) 261 nm (ϵ 1.63 × 10^4), 282 (sh) (1.06 × 10^4); (pH 6.9) 255 (1.62 × 10^4), 276 (sh) (1.17 × 10^4); (pH 13) 261 (1.43 × 10^4), 270 (sh) (1.37 × 10^4); NMR δ 3.60 (m, 2, H-5′), 3.74 (s, 3, OCH_3), 3.90 (m, 1, H-4′), 4.14 (m, 1, H-3′), 4.47 (m, 3, H-2′ + $ArCH_2$, changes shape on addition of D_2O), 4.87 (t, 1, OH-5′, exchanges with D_2O), 5.18 (d, 1, OH, exchanges with D_2O), 5.40 (d, 1, OH, exchanges with D_2O), 5.76 (d, 1, H-1′), 6.9 (t, 1, N^2H, exchanges with D_2O), 7.13 (q, 4, Ar), 7.95 (s, 1, 8-H), 10.60 (br s, 1, 1-NH, exchanges with D_2O). Anal. Calcd for $C_{18}H_{21}N_5O_6 \cdot H_2O$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.63; H, 5.36; N, 16.70.

Crystallization of the material recovered in fractions 80–100 from aqueous 1% KCl afforded 0.10 g (1%) of **6**: UV λ_{max} (pH 1) 258 nm (ϵ 1.74 × 10^4), 275 (sh) (1.33 × 10^4); (pH 6.9) 256 (1.89 × 10^4), 269 (sh) (1.50 × 10^4); (pH 13) 256 (1.78 × 10^4), 269 (sh) (1.41 × 10^4); NMR δ 3.58 (m, 2, H-5′), 3.72 (s, 3, OCH_3), 3.87 (m, 1, H-4′), 4.11 (m, 1, H-3′), 4.43 (m, 1, H-2′), 4.98 (br m, 2, OH + OH-5′, exchange with D_2O), 5.17 (s, 2, $ArCH_2$), 5.34 (br m, 1, OH, exchanges with D_2O), 5.71 (d, 1, H-1′), 6.97 (s, 2, NH_2, exchange with D_2O), 7.04 (q, 4, Ar), 7.98 (s, 1, H-8). Anal. Calcd for $C_{18}H_{21}N_5O_6 \cdot H_2O$: C, 51.30; H, 5.50; N, 16.62. Found: C, 51.23; H, 5.50; N, 16.36.

Purification of **4** required additional chromatographic procedures. The solid recovered by evaporation of LH-20 column fractions 51–60 was redissolved in 4 mL of H_2O and was loaded on a 0.72 × 30 cm Aminex A-5 column (NH_4^+ form) which was eluted with 0.3 M NH_4^+ HCO_2^- in CH_3CN/H_2O (3:7), pH 4.5, 40 °C, at 0.5 mL/min. When 50 mL of this solvent had passed through the column, elution was continued with 40 mL of CH_3CN/H_2O (4:6) and finally with $CH_3CN/H_2O/NH_3$–H_2O (5:4:1). Ultraviolet absorption was continuously monitored at 254 nm and fractions (1 mL) were collected. Product **4** eluted in 15 fractions after approximately 20 mL of this latter solvent had passed through the column: yield, 0.50 g (6%); UV λ_{max} (pH 1) 223 nm (ϵ 1.41 × 10^4), 274 (1.38 × 10^3), 280 (sh) (1.19 × 10^3); (pH 6.9) 222 (1.46 × 10^4), 274 (1.48 × 10^3), 280 (sh) (1.25 × 10^3); (pH 13) 222 (1.56 × 10^4), 242 (sh) (7.97 × 10^3), 272 (sh) (2.35 × 10^3), 280 (sh) (1.62 × 10^3); MS *m/z* 377 (M+), 245 (B + 1)$^+$, 121 (C_8H_9O); NMR δ 3.51 (m, 4, H-5′ + $ArCH_2$), 3.69 (s, 3, OCH_3), 3.76 (m, 1, H-4′), 4.02 (m, 1, H-3′), 4.21 (m, 1, H-2′), 5.16 (br s, 7, 4NH + 3OH, exchange with D_2O), 5.34 (d, 1, H-1′), 6.92 (q, 4, Ar), 7.42 (s, 1, H-2). Anal. Calcd for $C_{17}H_{23}N_5O_5$: C, 54.10; H, 6.14; N, 18.56. Found: C, 53.86; H, 6.30; N, 18.48.

Kinetics and Product Distributions. Rates of disappearance of **1** were determined spectrophotometrically by monitoring the time-dependent decrease in absorbance at 280 nm for solutions of **1** (1 × 10^{-4} M) in MeOH/H_2O (5:95 v/v) at 40 °C. Observed first-order rate constants (k_{obsd}) were determined from the slopes of ln (OD_t – OD_∞) vs. time plots and are the average of at least duplicate determinations. Buffers for the various pH conditions were prepared at similar ionic strengths ($\mu \simeq 0.2$).

Product determinations were performed after solutions of **1** (1.2 × 10^{-3} M) were allowed to incubate in MeOH/H_2O (5:95) at 40 °C for at least 5 half-times at a specific pH. Product separations were carried out by loading an aliquot (2 mL) of the resulting solutions of **1** on a 0.78 × 30 cm Aminex A-5 column (NH_4^+ form) which was initially eluted with 1 M NH_4^+ HCO_2^- in H_2O, pH 4.5, 40 °C, at 0.5 mL/min. Ultraviolet absorption was continuously monitored at 254 nm and fractions (1.0 mL) were collected. Guanosine (**2**) eluted in fractions 25–32. After 100 mL of this initial solvent had passed through the column, elution was carried out with 1 M NH_4^+ HCO_2^- in DMF/H_2O (15:85), pH 4.5, 40 °C. 8-(*p*-Methoxybenzyl)guanosine (**7**) eluted in fractions 122–130, 1-(*p*-methoxybenzyl)guanosine (**6**) eluted in fractions 135–143, and N^2-(*p*-methoxybenzyl)guanosine (**3**) eluted in fractions 145–160. After approximately 170 fractions were collected, column elution was carried out

(29) Weast, R. C. "Handbook of Chemistry and Physics", 56th ed.; CRC Press: Cleveland, Ohio, 1975; p C-516.

(30) Stephens, H.; Weizmann, C. *J. Chem. Soc.* **1914**, *105*, 1152.

with 1 M NH_4^+ HCO_2^- in DMF/H_2O (3:7), pH 6, 40 °C. 4-(*p*-Methoxybenzyl)-5-guanidino-1-β-D-ribofuranosylimidazole (**4**) eluted in fractions 203-210 and 7-(*p*-methoxybenzyl)guanosine (**5**) eluted in fractions 235-246. Fractions containing the individual products were pooled and the concentration of each product was determined spectrophotometrically.

Reaction of Radiolabeled Guanosine with *p*-Methoxybenzyl Chloride at pH 4.5. To a 2-mL solution of [5'-^{3}H]guanosine (21 Ci/mmol, 1×10^{-7} M) in HoAc/Na^+AcO^- buffer (0.1 M, pH 4.5) was added 0.05 mL of a freshly prepared solution of *p*-methoxybenzyl chloride (0.68 M) in DMF. The resulting suspension was stirred at 40 °C for 24 h. At the end of this incubation, 1 mL of the resulting solution was mixed with a solution of markers for **3**-**7** and was loaded on the Aminex A-5 column which was eluted as described above for separation of the unlabeled products. The eluted fractions were mixed with PCS (Amersham/Searle) for scintillation counting. Yields for products **3** and **5**-**7**, expressed as % guanosine converted to *p*-methoxybenzylated product were 3.4, 0.34, 0.05, and 0.07%, respectively.

Acknowledgment. This work was supported by Contract No. N01-CO-75380 with the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.

The Chemistry of 1-Carba-1-deaza-N^5-ethyl-N^3-methyllumiflavins. Influence of the N^1 upon the Reactivity of Flavin 4a-Hydroperoxides

Sheldon Ball and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. *Received January 28, 1981*

Abstract: N^5-Ethyl-N^3-methyl-1,5-dihydro-1-deazalumiflavin (C^1-FlEtH) has been synthetized and characterized. In aqueous solution (pH 3) C^1-FlEtH reacts with 1 equiv of $^3O_2$ to provide N^5-ethyl-N^3-methyl-1-deazalumiflavinium cation (C^1-Fl_{ox}^+Et). C^1-Fl_{ox}^+Et may be reduced to C^1-FlEtH by ascorbate, dithionite, or H_2/Pd. C^1-Fl_{ox}^+Et is not photoreducible by EDTA as is Fl_{ox}^+Et. This is due to direct photolysis of C^1-Fl_{ox}^+Et with the accompanying loss of the N^5-ethyl substituent as acetaldehyde. The spectral properties of C^1-$FlEtH_2^+$, C^1-FlEtH, and C^1-$FlEt^-$ and associated pK_a's have been determined and compared to the analogous constants for $FlEtH_2^+$, FlEtH, and $FlEt^-$. A comparison of the spectral properties of Fl_{ox}^+Et and C^1-Fl_{ox}^+Et has been made. The pK_a values and the pH dependences of the rate constants for the formation and dissociation of the pseudobases (i.e., C^1-4a-FlEtOH and 4a-FlEtOH) of Fl_{ox}^+Et and C^1-Fl_{ox}^+Et have also been determined as have the rate constants (pH 3.0) for addition of β-mercaptoethanol to the 4a-positions of Fl_{ox}^+Et and C^1-Fl_{ox}^+Et (providing 4a-FlEt-SCH_2CH_2OH and C^1-4a-FlEt-SCH_2CH_2OH). Partial oxidation of C^1-FlEtH by $^3O_2$ in H_2O produces the radical C^1-FlEt· through comproportionation of C^1-Fl_{ox}^+Et and C^1-FlEtH. Evidence is presented, suggesting that the radical C^1-FlEt· possesses a higher free-energy content than does FlMe·. The oxidation of C^1-FlEtH in H_2O or *t*-BuOH with excess $^3O_2$ is autocatalytic in nature. The initial rate for reaction of C^1-FlEtH with $^3O_2$ is substantially greater than the initial rate for reaction of FlMeH with $^3O_2$. This observation is discussed in terms of the mechanism of reaction of FlRH with O_2. In DMF, C^1-FlEtH reacts with $^3O_2$ to form a 4a-hydroperoxide (i.e., C^1-4a-FlEtOOH) which is quite stable. The rate constants for solvolysis of C^1-4a-FlEtOOH and 4a-FlEtOOH in DMF have been compared. The second-order rate constants for the (a) oxidation of I^- in 95% EtOH/DMF, (b) N-oxidations of *N*,*N*-dimethylbenzylamine, *N*-methylbenzylamine, and morpholine in DMF, and (c) the S-oxidation of thioxane in DMF by C^1-4a-FlEtOOH and 4a-FlEtOOH have been determined. The flavin products for the N- and S-oxygenation reactions are the pseudobases C^1-4a-FlEtOH and 4a-FlEtOH. These reactions are quantitative. Comparison of the various rate constants indicates that C^1-4a-FlEtOOH is from 3- to 17-fold a poorer oxidizing agent than is 4a-FlEtOOH. This can be explained by the somewhat less electronegative character of the 4a-position of the 1-deazaflavin hydroperoxide. The equilibrium constants for 4a-additions and retroadditions to C^1-Fl_{ox}^+Et and Fl_{ox}^+Et are comparable, and this leads to the conclusion that the difference in free-energy contents of C^1-Fl_{ox}^+Et and Fl_{ox}^+Et (starting states) and C^1-4a-FlEtX and 4a-FlEtX (products) is the same. Due to this feature, the decrease in $\Delta G^\ddagger$ for 4a additions to Fl_{ox}^+Et, as compared to C^1-Fl_{ox}^+Et (due to the greater electronegativity of Fl_{ox}^+Et), is mirrored in a decrease in $\Delta G^\ddagger$ for dissociation of X from 4a-FlEtX as compared to C^1-4a-FlEtX. This same free-energy difference is seen in the N- and S-oxidations and oxidation of I^- supporting the contention that the greater electrophilicity at the 4a-position of 4a-FlEtOOH polarizes the $C_{4a}O$-OH bond to a greater extent in 4a-FlEtOOH than in C^1-4a-FlEtOOH, thus making 4a-FlEtOOH a better oxidant. The observation that C^1-4a-FlEtOOH differs from 4a-FlEtOOH by only 1 order of magnitude in its oxygen-transfer potential to amines and the sulfide thioxane, combined with the established propensity of 4a-FlEtOOH to enter into these reactions, suggests that the hepatic flavoprotein microsomal oxidase reconstituted with 1-carba-1-deaza FAD will retain activity, if recognized by the enzyme, in the N-oxidation of amines and the S-oxidation of sulfides.

Introduction

Molecular oxygen is reductively activated by relatively few enzymatic systems. The flavoenzyme mono- and dioxygenases represent the only cofactor requiring oxygenases which do not employ a metal ion as a requisite component of their activities. A flavoprotein monoxygenase abundant in mammalian liver and located intracellularly on the endoplasmic reticulum near the sites of protein synthesis oxidatively metabolizes tertiary amine drugs to *N*-oxides, secondary amines to hydroxylamines, and sulfide pesticides to sulfoxides.[1] It has been established (for several flavin monooxygenases) that the reductive activation of molecular oxygen produces a transient enzyme-bound flavin 4a-hydroperoxide (Enz-4a-FlHOOH, eq 1), the reactive species in the N-oxidation of amines and the S-oxidation of sulfides.[2]

(1) (a) Ziegler, D. M.; Mitchell, C. H. *Arch. Biochem. Biophys.* **1972**, *150*, 116. (b) Hajjar, N. P.; Hodgson, E. *Science (Washington, D.C.)* **1980**, *209*, 1134.

0002-7863/81/1503-5494$01.25/0